

# ***HOXA5* is a tumor suppressor gene that is decreased in gastric cancer**

XUDONG PENG, LANG ZHA, ANQI CHEN and ZIWEI WANG

Gastrointestinal Surgical Unit, The First Affiliated Hospital of Chongqing Medical University, Chongqing 400000, P.R. China

Received November 29, 2017; Accepted May 31, 2018

DOI: 10.3892/or.2018.6537

**Abstract.** The abnormal expression of homeobox A5 (*HOXA5*) has been observed in breast and colon cancer; however, the clinical significance of *HOXA5* in gastric cancer (GC) is not yet clear. In this study, we found that *HOXA5* expression was decreased in GC tissues at the mRNA and protein level compared with paracancerous tissues using reverse transcription-quantitative PCR (RT-qPCR) and western blot analysis, respectively. Immunohistochemistry and Kaplan-Meier survival analysis confirmed that the under-expression of *HOXA5* was associated with GC progression and indicated a poor prognosis of patients with GC. Given that proliferation-related genes may be potential target genes of *HOXA5*, we performed a series of experiments *in vitro* to examine the effects of *HOXA5* on the proliferation of GC cells. A CCK-8 assay, colony formation assay and flow cytometry revealed that *HOXA5* inhibited the abnormal proliferation of GC cells, and this finding was further supported by a 5-ethynyl-2'-deoxyuridine (EdU) assay. Further mechanistic investigation clarified that *HOXA5* promoted the protein expression of p21 and inhibited the protein expression of c-Myc and Ki67. Additionally, the use of nude mouse models also verified that *HOXA5* suppressed the proliferation of GC cells *in vivo*. Collectively, the findings of this study demonstrate that *HOXA5* acts as a tumor suppressor gene during the development and progression of GC, possibly functioning by inhibiting the abnormal proliferation of cancer cells.

## **Introduction**

Gastric cancer (GC) is the second most common malignant tumor in terms of morbidity and mortality. There are 900,000 new cases of GC and 700,000-related deaths worldwide each

year (1). However, no biomarkers for GC are routinely used to predict its occurrence and prognosis.

Homeobox A5 (*HOXA5*) is located on chromosome 7, has a total length of 2,319 bp and is a member of the *HOX* family. Its encoded protein, *HOXA5*, contains 403 amino acids. *HOXA5* is a transcription factor that is involved in regulating human embryonic development and adult stem cell differentiation (2,3). The role of *HOXA5* in cancer progression has been gradually clarified. It has been demonstrated that *HOXA5* inhibits the activity of the Wntless (Wnt) pathway through the regulation of  $\beta$ -catenin inhibitory proteins and forms a negative feedback loop with Wnt signals to inhibit the stemness characteristics of colorectal cancer cells (4). Retinoic acid (RA) is a promising anticancer drug, and it has been found that the enforced overexpression of *HOXA5* induces the apoptosis and differentiation of breast cancer cells. Further mechanistic research has revealed that the RA response element upstream of the *HOXA5* promoter combines with RA receptor  $\beta$  (RAR $\beta$ ), thus mediating the anticancer effects of RA (5). F-actin reorganization and filopodia formation are known to promote cell movement, and *HOXA5* in lung cancer cells has been shown to decrease the level of myosin-X proteins expressed on filopodia tips and to reduce the number of pseudopods, thereby preventing cancer cell metastases. Survival analysis has indicated that *HOXA5* expression is associated with a better overall and disease-free survival of patients with non-small cell lung cancer expressing wild-type EGFR (6). These studies have suggested that *HOXA5* may be a broad-spectrum tumor suppressor gene with the potential for wide-ranging significance and applications.

A recent study found that *HOXA5* was significantly differentially methylated between GC tissues and adjacent non-cancer tissues (7). However, to the best of our knowledge, no information is available to date regarding the expression of *HOXA5* and its function in human GC. Thus, in this study, in order to explore the vital role of *HOXA5* in the tumorigenesis and progression of GC, the expression of *HOXA5* in 81 patients with GC was detected by immunohistochemistry, and the association between *HOXA5* expression and the clinicopathological characteristics of the patients with GC was analyzed. Additionally, the potential value of *HOXA5* as a prognostic indicator was assessed in patients with GC. Finally, the effects of *HOXA5* on cell proliferation were investigated *in vitro* and *in vivo*.

---

*Correspondence to:* Dr Ziwei Wang, Gastrointestinal Surgical Unit, The First Affiliated Hospital of Chongqing Medical University, 1 Youyi Road, Yuzhong, Chongqing 400000, P.R. China  
E-mail: wangziwei571@sina.com

**Key words:** homeobox A5, gastric cancer, prognosis, proliferation

## Materials and methods

**Patients and specimens.** A total of 81 pairs of GC tissues and matched paracancerous tissues ( $\geq 6$  cm away from the tumor) for immunohistochemistry and reverse transcription-quantitative PCR (RT-qPCR) were collected from patients with GC after surgical resection at the First Affiliated Hospital of Chongqing Medical University from January to October, 2009. Due to the long storage time, only 72 in 81 pairs of tissue samples were qualified for RT-qPCR. All patients had complete pathological parameters and follow-up information, including 53 males and 28 females with an average age of 64.1 years (range, 42-83 years). TNM staging was as follows: 5 cases of stage I, 23 cases of stage II, 48 cases of stage III and 5 cases of stage IV. In total, 30 pairs of GC and matched paracancerous specimens for western blot analysis were collected from patients with GC admitted to the First Affiliated Hospital of Chongqing Medical University from July to August, 2016. The patient cohort comprised 18 male and 12 female patients with an average age of 58.3 years (range, 31-83 years). All patients underwent total or subtotal gastrectomy and did not receive radiotherapy and chemotherapy prior to surgery. The use of human tissue samples and experimental protocols were approved by the Medical Ethics Review Committee of the First Affiliated Hospital of Chongqing Medical University and written informed consent was obtained from all patients.

**Cells and lentiviral transduction.** The human GC cell line, SGC7901, was obtained from the Chinese Academy of Sciences Shanghai Cell Bank (Shanghai, China) and cultured in RPM-1640 (HyClone, Shanghai, China) supplemented with 10% fetal bovine serum (HyClone) in an incubator with 5% CO<sub>2</sub> at 37°C. The overexpression vector pLV-HOXA5-GFP-puro, the control vector pLV-GFP-puro and polybrene were obtained from Hanbio Technology (Shanghai, China). The SGC7901 cells were seeded at  $1 \times 10^5$ /well in 6-well plates (1 ml/well). After 10 h, lentiviruses were added into the plates at a MOI of 40. Polybrene was added at a final concentration of 5  $\mu$ g/ml to each well. The culture media were replaced after 15 h. After 72 h, the cells were examined to determine the transduction efficiency under a fluorescence microscope (Olympus, Tokyo, Japan) and puromycin (Beyotime, Shanghai, China) was then added at a final concentration of 2  $\mu$ g/ml to each well. After selection for 2 weeks, puromycin was removed and the transduced cells were used in further experiments.

**Antibodies and reagents.** Rabbit anti-human HOXA5 (Cat. #ab82645), p21 (Cat. #ab227443) and c-Myc (Cat. #ab32072) antibodies were purchased from Abcam (Cambridge, UK). Rabbit anti-human Ki67 (Cat. #AP19895b) antibody was purchased from Abgent (San Diego, CA, USA). Rabbit anti-human GAPDH (Cat. #10494-1-AP), cyclin D1 (Cat. #12363-1-AP) and cyclin E (Cat. #11554-1-AP) antibodies and horseradish peroxidase (HRP)-conjugated goat anti-rabbit secondary antibody (Cat. #SA00001-15) were purchased from Proteintech (Wuhan, China). Immunohistochemistry-related reagents, western blot analysis-related reagents, CCK-8 reagents and DAPI were purchased from Beyotime (Shanghai, China). All reagents used in RT-qPCR were purchased from Takara (Dalian, China). The 5-ethynyl-2'-deoxyuridine (EdU)

proliferation assay kit was obtained from Ribobio (Guangzhou, China). All primers used for RT-qPCR were obtained from Sangon Biotech (Shanghai, China).

**RT-qPCR.** RT-qPCR was used to assess the differences in HOXA5 mRNA expression levels between the GC tissues and adjacent paracancerous tissues. Total RNA was extracted using TRIzol reagent (Takara, Dalian, China) and reverse transcribed into cDNA using the reverse transcription kit from Takara. The reverse transcription conditions were as follows: 37°C, 15 min; 85°C, 5 sec. Two-step RT-qPCR was performed using a SYBR-Green assay on a CFX96 PCR machine (Bio-Rad, USA) according to the instruction. The PCR conditions were as follows: Pre-denaturation at 95°C for 30 sec; 45 cycles of denaturation at 95°C for 5 sec and annealing at 60°C for 30 sec, extension at 65°C for 1 min. The data were analyzed using the  $\Delta\Delta C_q$  method (8). The primers used are shown in Table I.

**Western blot analysis.** The cancer tissues and matched paracancerous tissues were lysed using RIPA lysis buffer, and the protein concentration was measured using the BCA assay after the lysates were harvested by centrifugation (12,000 rpm) at 4°C. Subsequently, 20  $\mu$ g of protein samples were separated by electrophoresis on a 10% sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) and transferred onto polyvinylidene fluoride membranes. The membranes were incubated overnight at 4°C with HOXA5 antibody (1:1,000), p21 antibody (1:1,000), p53 antibody (1:1,000), cyclin D1 antibody (1:2,000), cyclin E antibody (1:500), c-Myc antibody (1:1,000) and Ki67 antibody (1:1,000) following blocking of the non-specific binding sites for 2 h in 5% non-fat milk. After washing with TBS-T, membranes were incubated with secondary antibody (1:2,000) at 37°C for 2 h. GAPDH was used as a loading control. An enhanced chemiluminescence kit (ECL) was used for visualizing immunobands and the signal intensity of each band was measured by Fusion software (Vilber Lourmat, Paris, France) to calculate protein levels.

**Immunohistochemistry.** Tissues were fixed by formalin and embedded in paraffin blocks, and a series of sections (4-mm-thick) were prepared. The tissue antigens were repaired by sodium citrate buffer (pH 6.0). The sections were incubated with rabbit anti-human HOXA5 antibody at 4°C overnight after non-specific binding was blocked with goat serum. The scoring criteria were as follows: 5 fields were randomly selected from each section under a light microscope (Olympus), and the percentage of positively stained cells to total cells was calculated. The sections were scored as follows: 0, <5%; 1, 5-25%; 2, 26-50%; 3, 51-75%; and 4, >75%. Additionally, the staining intensity was scored as follows: 0, no staining; 1, light yellow; 2, brownish yellow; and 3, brown. The total score was the sum of staining range and intensity, and was classified as low expression ( $\leq 3$  points) or high expression ( $\geq 4$  points).

**CCK-8 and colony-forming assays.** For the CCK-8 assay, the cells were seeded at  $2 \times 10^3$ /well in 96-well plates (200  $\mu$ l/well). After cell adherence, 20  $\mu$ l CCK-8 reagent were added to each well on days 0, 1, 2 and 3. Following incubation at 37°C for 1.5 h, the OD value at 450 nm was measured using a

Table I. The sequences of the PCR primers used in this study.

Gene	Primer
<i>GAPDH</i>	F: 5'-CTTTGGTATCGTGGAAGGACTC-3' R: 5'-GTAGAGGCAGGGATGATGTTCT-3'
<i>HOXA5</i>	F: 5'-AGCCACAAATCAAGGACACA-3' R: 5'-GCTCGCTCACGGAACATATG-3'
<i>E-cadherin</i>	F: 5'-TGGCTTCCCTCTTTTCATCTCC-3' R: 5'-TCATAGTTCCGCTCTGTCTTTGG-3'
<i>N-cadherin</i>	F: 5'-CGTGAAGGTTTGCCAGTGTGA-3' R: 5'-CCTGGCGTTCTTTATCCCG-3'
<i>Vimentin</i>	F: 5'-TCAATGTAAAGATGGCCCTTG-3' R: 5'-TGAGTGGGTATCAACCAGAGG-3'
<i>CD44</i>	F: 5'-TACTCTGCTGCGTTGTCTATTG-3' R: 5'-ACAACACCACGCCAGAGGA-3'
<i>EpCAM</i>	F: 5'-CGCAGCTCAGGAAGAATGTGT-3' R: 5'-AGCCATTCAATTCTGCCTTCAT-3'
<i>Lgr5</i>	F: 5'-GAGCTGCCTTCCAACCTCAG-3' R: 5'-CCCGCAAGACGTAACCTCCTC-3'
<i>CCND1</i>	F: 5'-ATGTTTCGTGGCCTCTAAGATGA-3' R: 5'-CAGGTTCCACTTGAGCTTGTTTC-3'
<i>CCNB1</i>	F: 5'-AATAAGGCGAAGATCAACATGGC-3' R: 5'-TTTGTTACCAATGTCCCAAGAG-3'
<i>Ki67</i>	F: 5'-ATCGTCCCAGGTGGAAGAGTT-3' R: 5'-ATAGTAACCAGGCGTCTCGTGG-3'
<i>MMP2</i>	F: 5'-GACATACATCTTTGCTGGAGAC-3' R: 5'-TTCAGGTAATAGGCACCCTT-3'
<i>MMP7</i>	F: 5'-CGGATGGTAAGCAGTCTAGGG-3' R: 5'-AGGTTGGATACATCACTGCATTAG-3'
<i>VEGFA</i>	F: 5'-CACACAGGATGGCTTGAAGA-3' R: 5'-AGGGCAGAATCATCACGAAG-3'
<i>VEGFR2</i>	F: 5'-CCAGATGACAACCAGACGGA-3' R: 5'-AGCCTTCAGATGCCACAGACTC-3'
<i>EGFR</i>	F: 5'-AGGCACGAGTAACAAGCTCAC-3' R: 5'-ATGAGGACATAACCAGCCACC-3'
<i>ABCC1</i>	F: 5'-GTGATGGCCATGAAGACCAAGA-3' R: 5'-GCCAGTCCCAGGCATAAAG-3'
<i>ABCG2</i>	F: 5'-TCTCTTCTTCTGACGACCAA-3' R: 5'-AAACCACACTCTGACCTGCTG-3'
<i>Caspase-3</i>	F: 5'-TACCAGTGGAGGCCGACTTC-3' R: 5'-GCACAAAGCGACTGGATGAAC-3'
<i>BCL2</i>	F: 5'-CTGCACCTGACGCCCTTACC-3' R: 5'-CACATGACCCACCGAACTCAAAGA-3'
<i>BECN1</i>	F: 5'-GGCTGAGAGACTGGATCAGG-3' R: 5'-CTGCGTCTGGGCATAACG-3'
<i>p21</i>	F: 5'-AGCGGAACAAGGAGTCAG-3' R: 5'-CGTTAGTGCCAGGAAAGAC-3'
<i>p53</i>	F: 5'-GCGTAAACGCTTCGAGATGTT-3' R: 5'-TTTTTATGGCGGGAAGTAGACTG-3'
<i>C-Myc</i>	F: 5'-ACACCCGAGCAAGGACGCGA-3' R: 5'-CGCGGGAGGCTGCTGGTTTTTC-3'
<i>β-catenin</i>	F: 5'-GAGTGCTGAAGGTGCTATCTGTC-3' R: 5'-CTGAACAAGAGTCCCAAGGAGA-3'

F, forward; R, reverse.

microplate reader (Thermo Fisher Scientific, Waltham, MA, USA), and the growth curve was then plotted. For the colony formation assay, the cells were seeded at 500/well in 6-well plates (1 ml/well) and cultured in complete medium. The culture medium was replaced every 4 days. After 15 days, the cells were fixed with 4% paraformaldehyde and stained with crystal violet (Beyotime, Shanghai, China) at room temperature for 10 min. Images were obtained to calculate the number of clones.

**Flow cytometry.** At least  $5 \times 10^5$  cells were suspended and fixed with 70% ethanol for 2 h. Propidium iodide (PI) was used to stain the cells and the cell cycle distribution was detected using a flow cytometer (BD Biosciences, San Jose, CA, USA).

**5-ethynyl-2'-deoxyuridine (EdU) assay.** The cells were seeded at  $2 \times 10^3$ /well in 96-well plates (200  $\mu$ l/well) and were treated with 50  $\mu$ M EdU for 2 h at 37°C. After washing with PBS and fixing with 4% paraformaldehyde for 30 min, the cells were permeabilized with 0.5% Triton X-100 for 5 min. The cells were then incubated with 100  $\mu$ l 1X Apollo reaction cocktail for 30 min. DAPI was then used to stain the nuclei for 15 min. Images were obtained under a fluorescence microscope (Olympus) to calculate the proliferation rate.

**Nude mouse models.** A total of 12 nude mice aged 4 weeks old (female, weighing 13 to 16 g) were obtained from the Animal Experimental Center of Chongqing Medical University and were maintained at  $24 \pm 2^\circ\text{C}$  under a 40-70% humidity with free access to food and water. The experiment was divided into SGC7901-NC group and SGC7901-HOXA5 group, with 6 mice in each group. When mice were 5 weeks old,  $2 \times 10^6$  cells suspended in 100  $\mu$ l PBS were injected subcutaneously into the left flank. The tumor volume was monitored by measuring diameters and calculated as  $L \times S^2/2$  (L indicates the long side and S indicates the short side) every 3 days. After 3 weeks, the nude mice were sacrificed by ether anesthesia combined with carbon dioxide asphyxiation. The flow rate of the gas was 20% of the chamber volume per minute and the death of the mice was judged according to breathing, heartbeat, nerve reflex and muscle relaxation after 5 min. The tumor tissues were stained with hematoxylin and eosin (H&E) for pathological confirmation. The animal experimental protocols were approved by the Medical Ethics Review Committee of the First Affiliated Hospital of Chongqing Medical University.

**GEPIA analysis.** Gene Expression Profiling Interactive Analysis (GEPIA, <http://gepia.cancer-pku.cn>), an online cancer microarray database, was used to analyze the effect of HOXA5 on the overall survival of GC patients (9). Patients were divided into high expression group and low expression group according to the median expression level of HOXA5 mRNA (Normalized by GAPDH) and the overall survival was assessed by a Kaplan-Meier survival plot.

**Statistical analysis.** All experiments were repeated 3 times and the data were analyzed using SPSS 19.0 software. Measurement data are expressed as the means  $\pm$  standard deviation (SD). Comparisons were made between two groups using the Student's t-test. Wilcoxon signed-rank tests were used

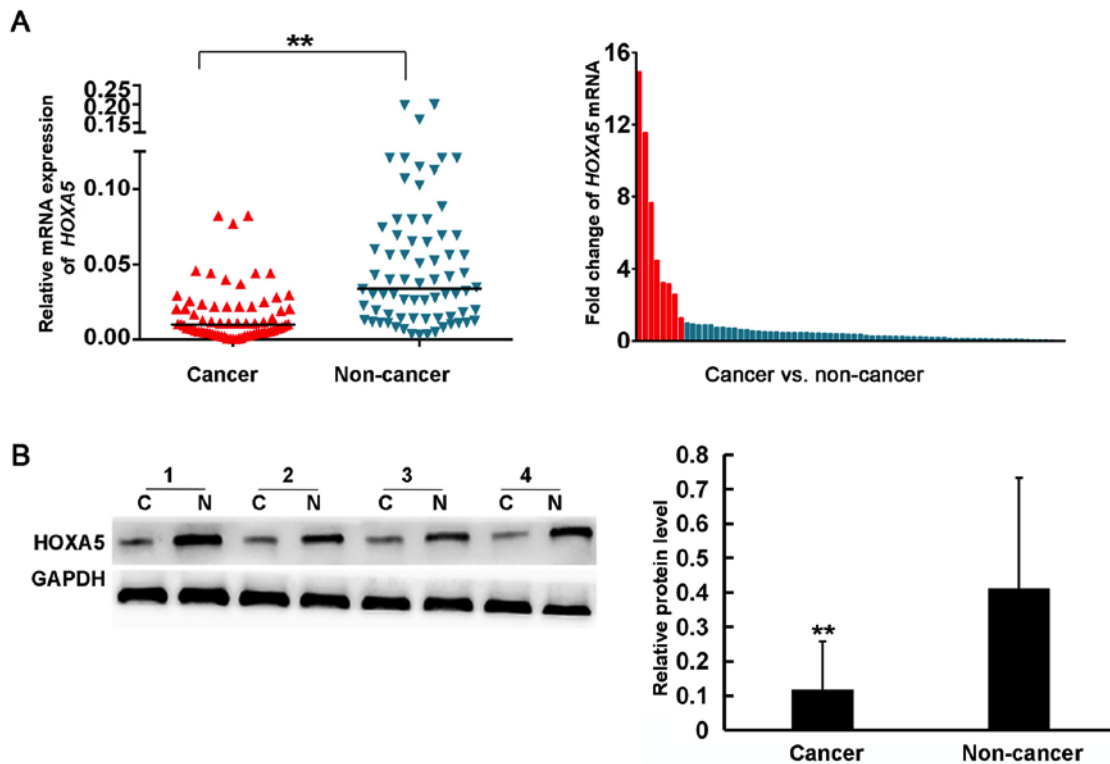


Figure 1. Homeobox A5 (*HOXA5*) expression is downregulated in gastric cancer (GC) tissues. (A) The mRNA expression levels of *HOXA5* in GC and adjacent non-cancer tissues of 72 patients were detected by RT-qPCR. *GAPDH* was used as an endogenous control (left panel), \*\* $P < 0.01$ ; The fold change is expressed as the ratio of *HOXA5* mRNA expression in GC tissue to the expression of non-cancer tissues (right panel). (B) The protein expression of *HOXA5* in GC tissues and adjacent non-cancer tissues was detected by western blot analysis.

to assess the differences in *HOXA5* mRNA expression levels between the GC tissues and adjacent paracancerous tissues of 72 patients. The correlation between *HOXA5* protein expression and each clinicopathological parameter was examined using  $\chi^2$  tests. Survival curves for different expression levels of *HOXA5* were plotted according to the Kaplan-Meier method, and prognostic significance of all factors was calculated by the log-rank test. Significant parameters ( $P < 0.05$ ) in the univariate model were further evaluated using multivariate Cox regression, after which the independence and hazard ratio (HR) of each prognostic factor could be calculated. Differences were considered statistically significant at a  $P$ -value  $< 0.05$ . The correlation between the expression of *HOXA5* mRNA and 23 genes related to malignant biological behavior in 30 GC tissues was determined using Spearman's correlation analysis.

## Results

**Decreased expression of *HOXA5* in human GC tissues.** To examine the role of *HOXA5* in GC, RT-qPCR was used to detect the mRNA expression of *HOXA5* in 72 pairs of GC issues and adjacent paracancerous tissues. The results demonstrated that 63 patients (87.5%) exhibited a lower mRNA expression of *HOXA5* in GC tissues compared to the matched non-cancer tissues (Fig. 1A). The average mRNA expression level of *HOXA5* in the cancer tissues was 3.23-fold lower than that in the adjacent non-cancer tissues. Subsequently, western blot analysis was performed to determine whether *HOXA5* was also downregulated at the protein level in 30 GC patients. As shown in Fig. 1B, the average protein expression level of

*HOXA5* in the GC tissues was 3.73-fold lower than that in the non-cancer tissues ( $0.11 \pm 0.14$  vs.  $0.41 \pm 0.32$ , respectively). These results suggested that *HOXA5* expression was decreased in GC.

***HOXA5* acts as a tumor suppressor gene in GC.** To further illuminate the role of *HOXA5* in the progression of GC, we used immunohistochemistry to detect the expression of *HOXA5* in samples from 81 patients with GC and analyzed the association between *HOXA5* and clinical parameters. We found that *HOXA5* staining was mainly located in the nucleus and cytoplasm (Fig. 2). Compared with a 75.3% positive expression of *HOXA5* (61/81) in the adjacent non-cancer tissues, the positive expression in the GC tissues was only 35.8% (29/81).

The GC specimens were then divided into a high expression group and a low expression group, according to the immunohistochemistry score for *HOXA5* expression. As shown in Table II, a decreased *HOXA5* expression was significantly associated with tumor size and histological grade. These results thus suggest that *HOXA5* acted as a tumor suppressor gene in the progression of GC.

**Decreased expression of *HOXA5* protein as a prognostic marker for GC.** To investigate the prognostic value of *HOXA5* for GC, survival analysis from GEPIA database was obtained. *HOXA5* mRNA was not associated with the overall survival of patients with GC (Fig. 3A). Given that *HOXA5* functions as a transcription factor, we further assessed the association between *HOXA5* protein expression and survival according



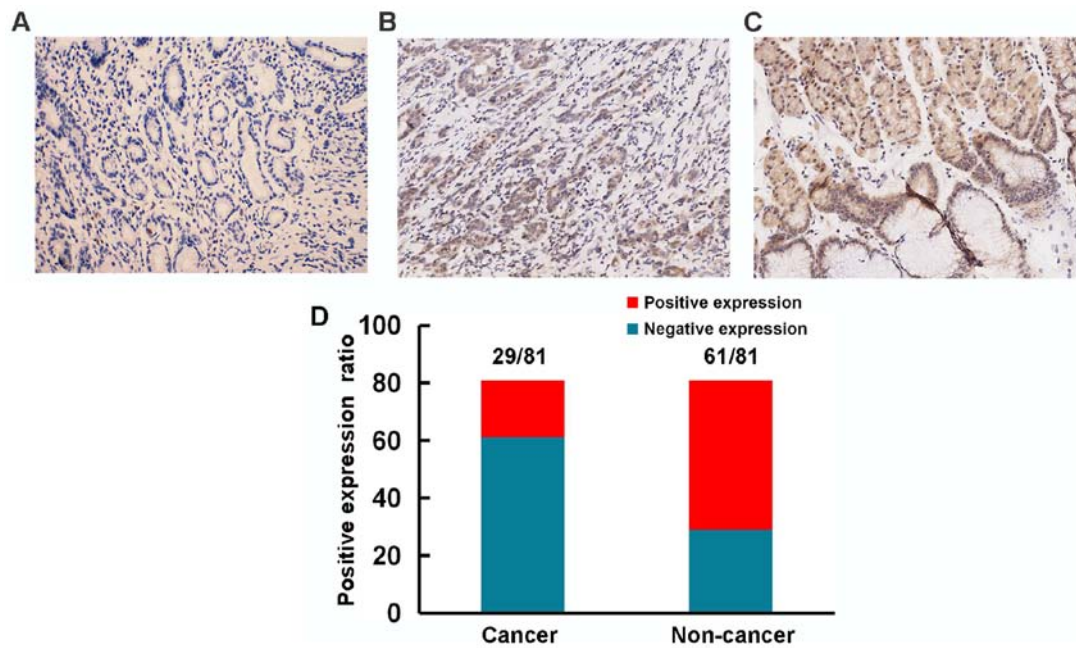


Figure 2. Representative immunohistochemical images showing the expression of homeobox A5 (HOXA5) in paraffin-embedded sections (magnification, x200). (A) A gastric cancer sample without HOXA5 expression. (B) A gastric cancer sample with a high expression level of HOXA5. (C) A non-cancer sample with a high expression level of HOXA5. (D) The positive HOXA5 staining ratio in GC samples and adjacent non-cancer samples.

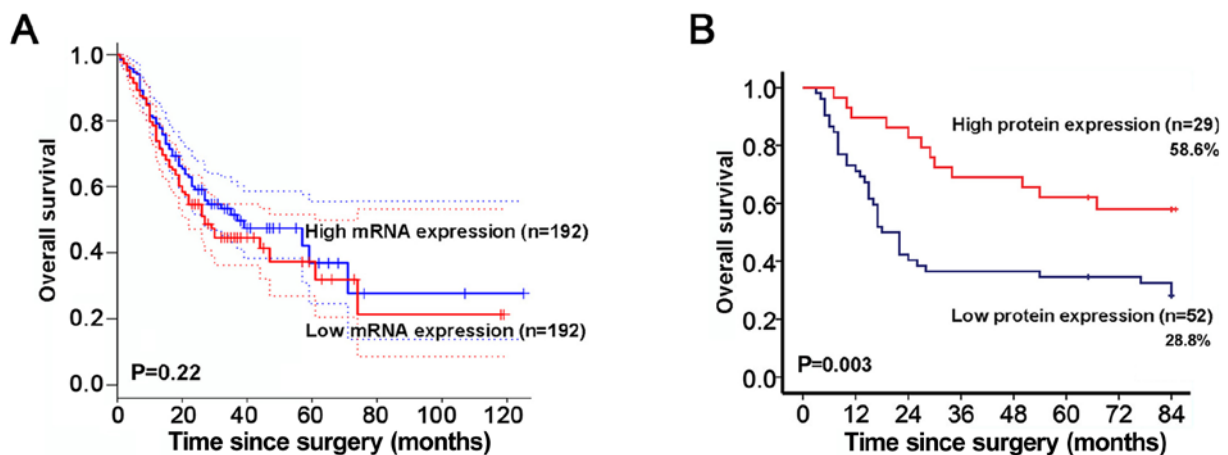


Figure 3. Decreased expression of homeobox A5 (HOXA5) protein indicates a poor prognosis of patients with gastric cancer (GC). (A) The overall survival of patients with GC was compared between the patients with a high *HOXA5* mRNA expression and those with a low *HOXA5* mRNA expression by Kaplan-Meier survival curve analysis. (B) The overall survival of patients with GC was estimated by Kaplan-Meier survival curve analysis and compared between the patients with high *HOXA5* protein expression and those with low *HOXA5* protein expression using the log-rank test.

to the immunohistochemistry score. It is worth noting that the 75% survival duration of the patients in the low protein expression group was 10 months, which was significantly shorter than that of 30 months for the patients in the high protein expression group. Patients with a low protein expression level of HOXA5 had an overall survival rate of 28.8% in comparison to 58.6% for patients with a high protein expression level (Fig. 3B).

According to a univariate analysis of the prognostic factors of GC, the overall survival was found to be significantly associated with each of the following: Histological grade ( $P=0.031$ ), tumor size ( $P<0.001$ ), lymph node involvement ( $P<0.001$ ), TNM stage ( $P<0.001$ ), distant metastasis ( $P<0.001$ ), and decreased HOXA5 expression ( $P=0.003$ ). To investigate

whether a decreased HOXA5 is an independent prognostic marker in patients with GC, a multivariate Cox regression analysis was performed. We found that tumor size, lymph node involvement, TNM stage, distant metastasis and a decreased HOXA5 expression were significantly associated with overall survival after controlling for other prognostic factors in the multivariate Cox regression analysis (Table III). These data suggested that a decreased HOXA5 expression was an independent risk factor for the prognosis of GC and could be a prognostic marker for patients with GC.

*Identification of potential target genes of HOXA5.* The progression of GC involves a series of cellular biological events, including the loss of control of cell

Table II. Association between the expression of HOXA5 and clinical parameters of patients with gastric cancer (GC).

Index	Case	HOXA5		$\chi^2$	P-value
		+	-		
Sex				0.974	0.324
Male	53	21	32		
Female	28	8	20		
Age (years)				0.822	0.365
<60	31	13	18		
≥60	50	16	34		
Histological grade				7.814	0.005 <sup>b</sup>
Well-differentiated	39	20	19		
Poorly-differentiated	42	9	33		
Tumor size (cm)				10.65	0.001 <sup>b</sup>
<5	39	21	18		
≥5	42	8	34		
TNM stage				2.486	0.115 <sup>a</sup>
1+2	38	17	21		
3+4	43	12	31		
Lymph node involvement				0.510	0.475
No	24	10	14		
Yes	57	19	38		
Vascular invasion				2.523	0.112
Absent	16	3	13		
Present	65	26	39		
Distant metastasis				0.008	0.928
No	78	28	50		
Yes	3	1	2		

<sup>a</sup>P<0.05, <sup>b</sup>P<0.01; Chi-square test.

Table III. Kaplan-Meier univariate survival analysis and multivariate Cox regression analysis of prognostic factors in gastric cancer (GC) for overall survival.

Variables	Univariate	Multivariate analysis		
	P-value	HR	95% CI	P-value
Sex	0.307			
Age (years)	0.943			
Histological grade	0.031 <sup>a</sup>	0.683	0.379-1.234	0.206
Tumor size	<0.001 <sup>b</sup>	2.697	1.309-5.558	0.007 <sup>b</sup>
TNM stage	<0.001 <sup>b</sup>	3.083	1.474-6.447	0.003 <sup>b</sup>
Lymph node	<0.001 <sup>b</sup>	3.787	1.308-10.962	0.014 <sup>a</sup>
Vascular invasion	0.284	0.714	0.334-1.526	0.385
Distant metastasis	<0.001 <sup>b</sup>	9.263	2.068-41.502	0.004 <sup>b</sup>
HOXA5 expression	0.003 <sup>b</sup>	0.429	0.202-0.912	0.028 <sup>a</sup>

<sup>a</sup>P<0.05, <sup>b</sup>P<0.01; log rank test and cox regression.

proliferation, an enhanced angiogenesis, the acquisition of epithelial-mesenchymal transition (EMT) and stemness, and sustained invasion and drug resistance. Our data suggested

that HOXA5 inhibited the progression of GC. In order to further explore the underlying mechanisms, we used RT-qPCR to detect the expression of the following genes in

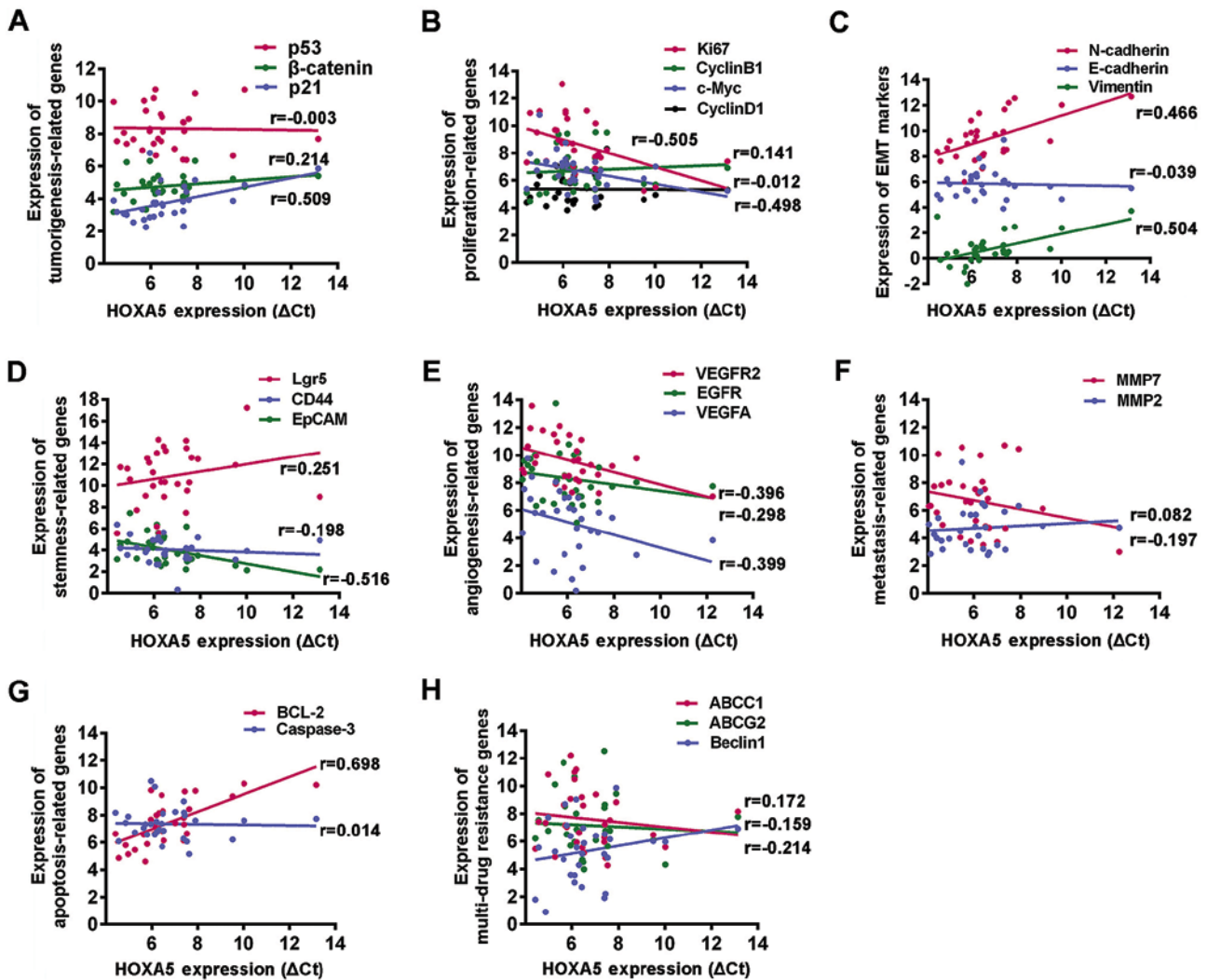


Figure 4. Correlation between homeobox A5 (*HOXA5*) and cancer malignant behavior-related genes. *HOXA5* and the mRNA expression of 23 typical malignant behavior-related genes were detected using RT-qPCR in 30 GC tissues. *GAPDH* was used as a loading control and the relative expression levels were expressed by  $\Delta\Delta Cq$ . Spearman's correlation analysis was used to determine the correlation *HOXA5* and (A) tumorigenesis-related genes, (B) proliferation-related genes, (C) EMT markers, (D) stemness markers, (E) angiogenesis-related genes, (F) metastasis-related genes, (G) apoptosis-related genes, and (H) multi-drug resistance genes.

30 GC tissues, including tumorigenesis-related genes (*p21*, *p53* and  $\beta$ -*catenin*), proliferation-related genes (*cyclin B1*, *cyclin D1*, *c-Myc* and *Ki67*), angiogenesis-related genes [vascular endothelial growth factor (*VEGF*)A, *VEGFR2* and epidermal growth factor (*EGFR*)], metastasis-related genes [matrix metalloproteinase (*MMP2*) and (*MMP7*)], apoptosis-related genes (*BCL2* and *caspase-3*), EMT markers (vimentin, *N-cadherin* and *E-cadherin*), stemness markers (*Lgr5*, *CD44* and *EpCAM*) and multi-drug resistance genes (*ABCC1*, *ABCG2* and *Beclin1*) (10-15). A Spearman's correlation model was used to examine the correlation between each of these genes and *HOXA5* mRNA expression. We found that *HOXA5* mRNA expression positively correlated with the tumor suppressor gene, *p21*, and negatively correlated with the proliferation-related genes, *c-Myc* and *Ki67*, the angiogenesis genes, *VEGFA* and *VEGFR2*, and the stemness marker, *EpCAM*. No correlation was observed with the other genes examined. Surprisingly, although *HOXA5* exhibited a role in inhibiting the progression of GC, its mRNA expression positively correlated with the mesenchymal factors, *vimentin*

and *N-cadherin*, which indicated the acquisition of EMT; it also positively correlated with the anti-apoptotic molecule, *BCL2*, which is known to play an important role in apoptosis resistance (Fig. 4 and Table IV).

**Enforced overexpression of *HOXA5* suppresses the aberrant proliferation of GC cells.** The immunohistochemistry results revealed that *HOXA5* expression negatively correlated with the size of the tumor. Moreover, correlation analysis revealed that *Ki67*, *c-Myc* and *p21* (these 3 genes are involved in the regulation of tumor proliferation) may be potential target genes of *HOXA5*. This indicated that *HOXA5* may exert its anticancer effects through the negative regulation of cancer cell proliferation. To examine the function of *HOXA5* in the progression of GC, we used *HOXA5* overexpression vectors to transfect GC SGC7901 cells, which express a low level of *HOXA5*, and detected its effect on cell proliferation using a CCK-8 assay (Fig. 5A). The growth curve indicated that *HOXA5*-overexpressing cells (SGC7901-*HOXA5*) exhibited a significantly decreased proliferation compared with the

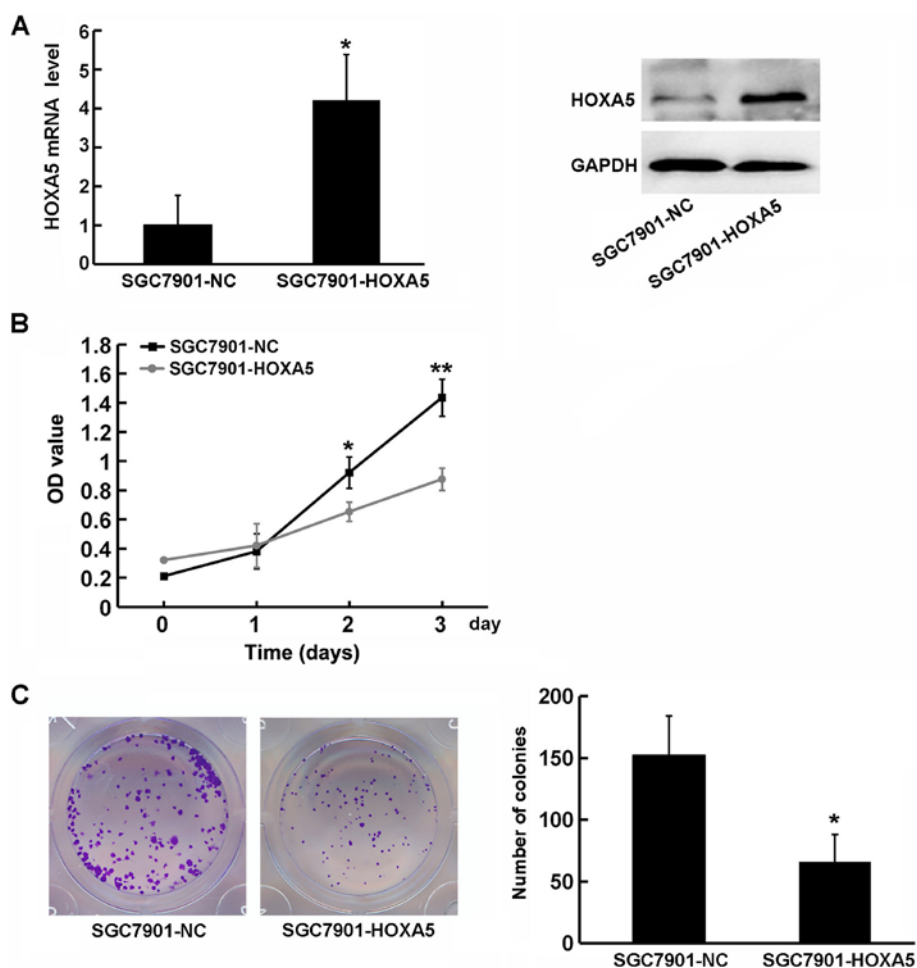


Figure 5. Homeobox A5 (HOXA5) attenuates the aberrant proliferation of gastric cancer (GC) cells. (A) SGC7901 cells were transduced with lentiviruses containing HOXA5 overexpression plasmids. After 72 h, the overexpression efficiency was confirmed by RT-qPCR (left panel) and western blot analysis (right panel). (B) The growth curves of SGC7901-NC and SGC7901-HOXA5 cells were plotted according to the CCK-8 assay. (C) A colony formation assay was used to investigate the colon formation ability of SGC7901-NC and SGC7901-HOXA5 cells.

negative control cells (SGC7901-NC) (Fig. 5B). Similarly, a colony formation assay revealed that HOXA5 overexpression resulted in an impairment of SGC7901 colony formation (Fig. 5C). These results indicated that the upregulation of HOXA5 in GC cells was beneficial in inhibiting cell growth, and that HOXA5 suppressed the proliferation of GC cells.

*Enforced overexpression of HOXA5 decelerates the  $G_1$ -S phase transition of GC cells.* Cell proliferation depends on the proper progression of the cell cycle, particularly in the  $G_1$ -S phase transition. As shown in Fig. 6A, the results from flow cytometry revealed that compared with the SGC7901-NC cells, the SGC7901-HOXA5 cells exhibited a significantly increased percentage of cells in the  $G_0/G_1$  phase (38.7% vs. 50.8%), but an decreased percentage of cells in the S phase (48.6% vs. 32.7%). We then employed an EdU staining assay to detect the cell proliferation rate of the cells in the SGC7901-NC and SGC7901-HOXA5 groups. Fluorescence microscopy revealed a lower proliferative activity in the SGC7901-HOXA5 group (19.2%) than that noted in the SGC7901-NC group (55.7%), which was consistent with the flow cytometry data (Fig. 6B). These results indicated that the enforced overexpression of HOXA5 decelerated the  $G_1$ -S transition of the GC cells.

*HOXA5 regulates the expression of cell cycle-related proteins.* Our data suggested that HOXA5 decelerated the cell cycle, thus inhibiting the proliferation of GC cells. In order to further clarify the underlying mechanisms, we used western blot analysis to detect the effect of HOXA5 on a series of cell cycle-related proteins. We found that HOXA5 promoted the expression of p21 and inhibited the expression of c-Myc and Ki67 at the protein level, although it had no marked effect on the expression of p53, cyclin D1 and cyclin E (Fig. 7). This may be the underlying mechanism through which HOXA5 exerts its inhibitory effect on GC cell proliferation.

*HOXA5 suppresses the proliferation and promotes the differentiation of GC cells in vivo.* Xenograft experiments *in vivo* are the gold standard to determine cell proliferation. Thus, we injected SGC7901-NC or SGC7901-HOXA5 cells subcutaneously into nude mice to observe their different effects on proliferation. As shown in the tumor growth curve, the SGC7901-HOXA5 group began to display a smaller tumor volume than that of the SGC7901-NC group from day 15 (Fig. 8A). In addition, H&E staining revealed that compared with the NC group, the tumors in the SGC7901-HOXA5 group were loosely arranged, with more mesenchymal and inflammatory cell infiltration and



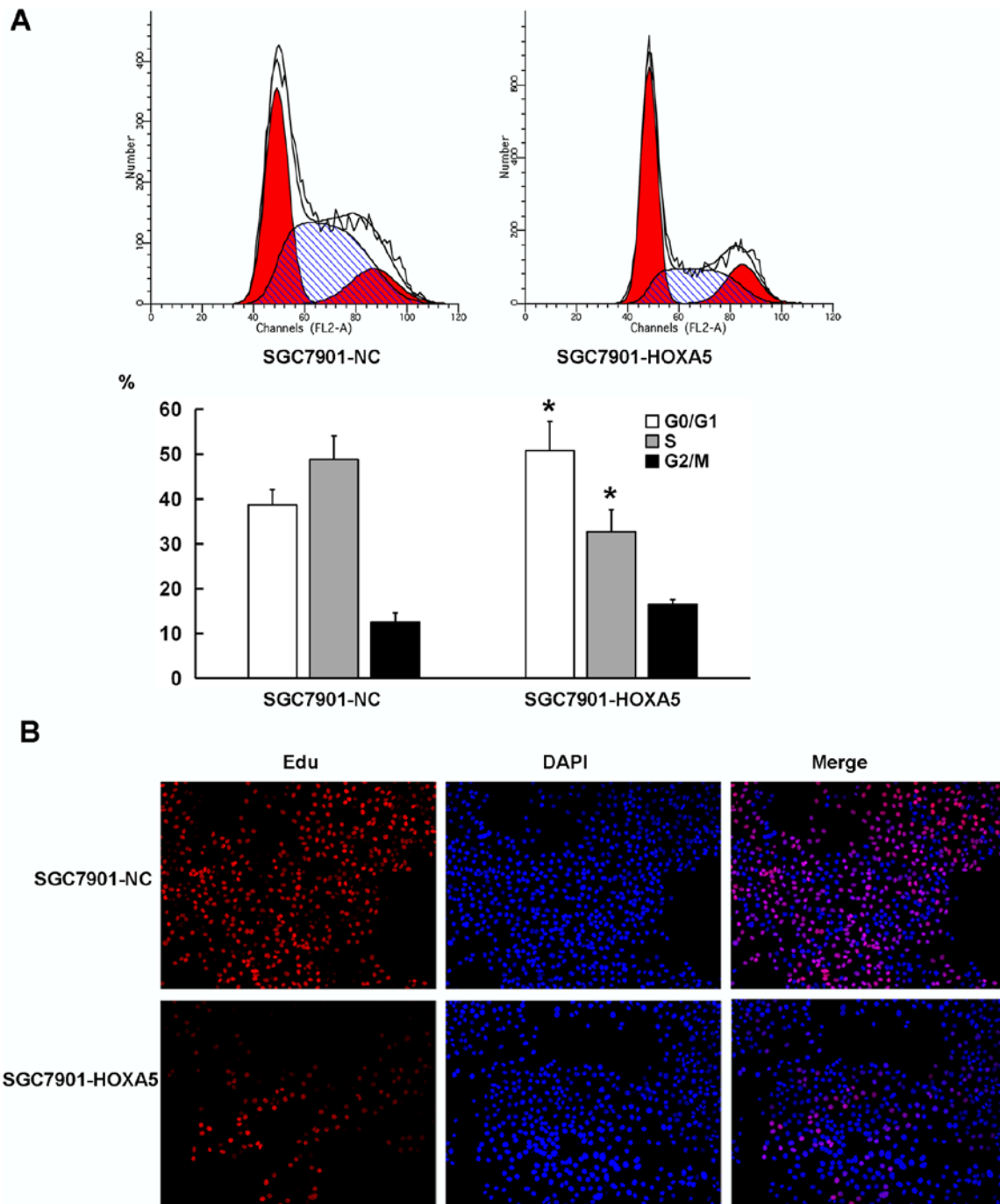


Figure 6. Effect of homeobox A5 (HOXA5) on cell cycle progression in gastric cancer (GC) cells. (A) The cell cycle distributions of SGC7901-NC and SGC7901-HOXA5 cells were evaluated by flow cytometry. Representative images of cell cycle distribution (high) and cell cycle analysis (low). (B) EdU staining was performed to determine the cell proliferation rate of SGC7901-NC and SGC7901-HOXA5 cells (magnification, x200). The proliferation rate was calculated using EdU-stained cell (red) number compared to the total cell number (DAPI-stained, blue). \* $P < 0.05$ .

fewer abnormal cell mitoses. The overexpression of HOXA5 promoted the tumor cells to enter a more differentiated state (Fig. 8B). These results suggested that HOXA5 suppressed the proliferation and promoted the differentiation of GC cells *in vivo*.

## Discussion

In the present study, we demonstrated that HOXA5 was down-regulated in GC and that the underexpression of HOXA5 was

associated with the progression of GC. In addition, we also demonstrated that the underexpression of HOXA5 was an independent prognostic factor for patients with GC. Furthermore, we revealed that HOXA5 may exert its anticancer effects through the inhibition of the abnormal proliferation of GC cells.

GC is the second most common malignant tumor worldwide and is associated with a poor prognosis. Despite the existence of comprehensive treatments based on surgery and chemotherapy, the 5-year survival rate of patients with

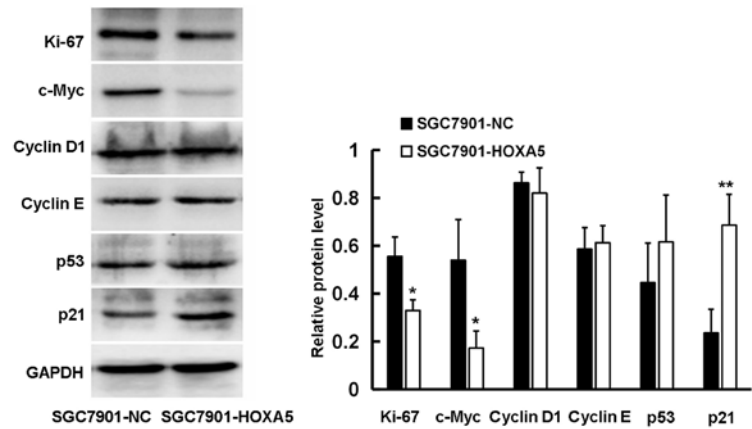


Figure 7. Homeobox A5 (HOXA5) regulation of cell cycle-related proteins. The protein expression levels of p21, p53, cyclin D1, cyclin E, c-Myc and Ki67 in SGC7901-NC and SGC7901-HOXA5 cells were detected by western blot analysis. GAPDH was used as a loading control. \*P<0.05, \*\*P<0.01.

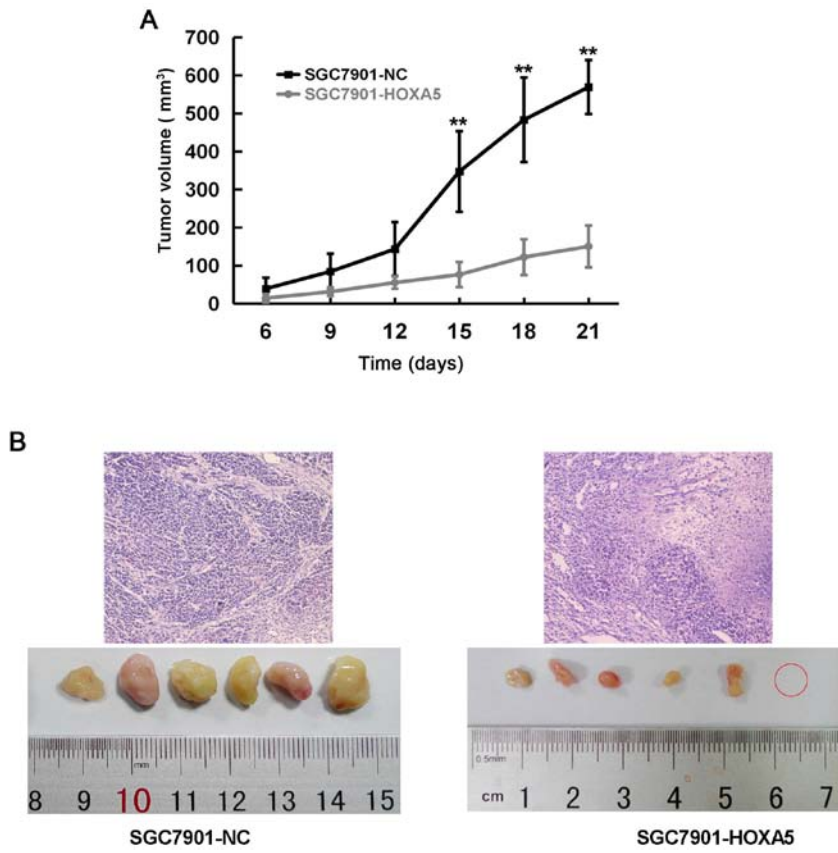


Figure 8. *In vivo* assessment of homeobox A5 (HOXA5) on the proliferation of gastric cancer (GC) cells. (A) Cells (2x10<sup>6</sup>) were subcutaneously injected into the left flank of nude mice, with 6 mice in each group. The tumor growth curve was plotted on the basis of the tumor volume monitored every 3 days. (B) After 3 weeks, the mice were sacrificed and the tumor tissues were stained for hematoxylin and eosin (H&E) for pathology confirmation. \*\*P<0.01.

GC has not improved significantly. It is thus of great clinical significance to find an effective molecular target for the early diagnosis and treatment of GC.

*HOXA5* is a member of the homeobox gene family, and it encodes the 29 kDa *HOXA5* protein, which functions as a critical master regulatory factor in controlling embryonic development and adult stem cell differentiation. Recently, accumulating evidence has revealed that the human *HOXA5* protein limits the aggressiveness of breast cancer and colon cancer (4,5). To clarify the associatoin between *HOXA5* and

GC, we detected the expression of *HOXA5* using RT-qPCR and western blot analysis in 30 patients with GC and found that *HOXA5* expression was significantly lower in GC tissues than that in adjacent non-cancer tissues. This result was consistent with a previous finding, in that *HOXA5* was hypermethylated in GC tissues (7). A recent study reported that *HOXA5* was a marker of a good prognosis in patients with colon cancer (4), although another study demonstrated that the overexpression of *HOXA5* was associated with a poor prognosis in non-small cell lung cancer (16). In this study, we

Table IV. Spearman's correlation analysis of the correlation between the expression of *HOXA5* and malignant behavior-related genes.

Variables	Spearman's correlation analysis	
	r value	P-value
Carcinogenesis-related genes		
<i>p21</i>	0.509	<0.01 <sup>b</sup>
<i>p53</i>	-0.003	0.987
<i>β-catenin</i>	0.214	0.256
Proliferation-related genes		
<i>CCNB1</i>	0.141	0.456
<i>CCND1</i>	-0.012	0.951
<i>Ki67</i>	-0.505	<0.01 <sup>a</sup>
<i>C-Myc</i>	-0.498	<0.01 <sup>a</sup>
EMT markers		
<i>E-cadherin</i>	-0.039	0.836
<i>N-cadherin</i>	0.466	0.01 <sup>a</sup>
<i>Vimentin</i>	0.504	<0.01 <sup>a</sup>
Stemness markers		
<i>Lgr5</i>	0.251	0.181
<i>CD44</i>	-0.198	0.295
<i>EpCAM</i>	-0.516	<0.01 <sup>a</sup>
Angiogenesis-related genes		
<i>VEGFA</i>	-0.399	0.029 <sup>b</sup>
<i>VEGFR2</i>	-0.396	0.03 <sup>b</sup>
<i>EGFR</i>	-0.298	0.109
Metastasis-related genes		
<i>MMP2</i>	0.082	0.668
<i>MMP7</i>	-0.197	0.296
Apoptosis-related genes		
<i>BCL2</i>	0.698	<0.01 <sup>a</sup>
<i>Caspase-3</i>	-0.014	0.941
Multi-drug resistance genes		
<i>ABCC1</i>	-0.214	0.256
<i>ABCG2</i>	-0.159	0.401
<i>Beclin1</i>	0.172	0.364

<sup>a</sup>P<0.05, <sup>b</sup>P<0.01; Spearman's correlation analysis.

found that a decreased *HOXA5* expression in GC tissues was associated with larger tumors that were poorly differentiated and a higher TNM stage.

Although that the mRNA level of *HOXA5* was not directly associated with the prognosis of patients with GC, patients with a low *HOXA5* protein expression had a significantly shorter overall survival than patients with a high expression. One possible explanation for this is that *HOXA5* is a transcription factor which functions as a protein and there may be post-transcriptional modifications during the process of translation of *HOXA5* mRNA. Further multivariate analysis confirmed that a decreased *HOXA5* expression, together with tumor size, lymph node involvement, distant metastasis and

TNM stage, were independent prognostic factors in patients with GC. Therefore, our results support the notion that a decreased *HOXA5* expression promotes the progression of GC and is an indicator of a poor prognosis of patients with GC.

As is widely known, cancer progression involves a series of cellular biological events, including the loss of control of cell proliferation, angiogenesis, EMT, stemness, invasion and drug resistance (17-20). In this study, we found that the mRNA expression of *HOXA5* positively correlated with the broad spectrum tumor suppressor gene, *p21*. Although it has been shown that *HOXA5* promotes apoptosis by the transcriptional regulation of *p53* in breast cancer cells expressing wild-type *p53* (21), our study did not show an association of *HOXA5* with *p53*. In addition, *HOXA5* was found to negatively correlate with the proliferation-related genes, *c-Myc* and *Ki67*, the angiogenesis-related genes, *VEGFA* and *VEGFR2*, and the stemness marker *EpCAM*, suggesting that *HOXA5* may suppress GC progression by inhibiting abnormal proliferation, angiogenesis and the acquisition of stemness. These results are consistent with previous findings on breast cancer and colon cancer (4,5). It is worth noting that *HOXA5* exhibited a significant positive correlation with the mesenchymal factors, *N-cadherin* and *vimentin*, and the anti-apoptotic molecule, *BCL2*. It is well known that the upregulation of mesenchymal molecules can induce cytoskeletal remodeling, polarity loss and an increased migratory capacity, which are early markers of metastasis in epithelium-derived tumors (22). However, taking into account that *HOXA5* is expressed in the mesenchyme and regulates organ patterning through signal pathways such as the hedgehog (Hh) and transforming growth factor- $\beta$  (TGF- $\beta$ ) pathways in the processes of respiratory and digestive tract development, it is not difficult to envision that *HOXA5* also increases *N-cadherin* and *vimentin* expression in GC (23). Although *HOXA5* may increase *BCL2* expression according to our data, the association between *HOXA5* and apoptosis remains to be further verified given the presence of multiple complex signaling pathways in the regulation of apoptosis. Our results suggested that *HOXA5* may inhibit the proliferation, angiogenesis and the stemness of GC through mediators or signaling pathways. Despite possibly facilitating EMT, *HOXA5* exerted an inhibitory effect on the progression of GC.

Previous studies have reported that *HOXA5* inhibits the proliferation of breast and lung cancer cells (24,25). Moreover, in this study, the results from immunohistochemistry also indicated that *HOXA5* may exert its anticancer effect through the negative regulation of cancer cell proliferation. To further confirm the role of *HOXA5* in GC cell proliferation, we over-expressed *HOXA5* by the transduction of lentiviral vectors in SGC7901 cells. As was suspected, the enforced overexpression of *HOXA5* significantly inhibited the proliferation of GC cells, as shown by CCK-8 and colony formation assays. The results from flow cytometry and EdU assay revealed that *HOXA5* overexpression induced arrested cell cycle at the G<sub>1</sub> phase. The G<sub>1</sub>-S transition is regulated by a series of factors (26,27).

In this study, we demonstrated that *HOXA5* upregulated *p21* and downregulated *c-Myc* and *Ki67*, although the expression of two important G<sub>1</sub> phase proteins, cyclin D1 and cyclin E, was not altered. The cyclin-dependent kinase inhibitor, *p21*, inhibits the activity of the cyclin D1-CDK4 and

cyclin E-CDK2 complexes and prevents the cells from entering the S phase from the G<sub>1</sub> phase, which is the most important pathway to block the G<sub>1</sub>-S transition (28,29). By contrast, the proliferation-related proteins, c-Myc and Ki67, contribute to the progression of the cell cycle. It is worth noting that the correlation model in tissues revealed that the correlation between these three genes and *HOXA5* was unimpressive. Our function assays in cells demonstrated that the overexpression of *HOXA5* can cause significant p21 upregulation and Ki67, c-Myc downregulation. One possible explanation is that *HOXA5* is a type of transcription factor and can function through downstream signaling pathways (27), or direct transcriptional activation or inhibition of target genes (30) in the form of a protein. Once *HOXA5* protein in the GC cells was upregulated, the downstream target genes revealed obvious changes. Therefore, we hypothesized that *HOXA5* suppressed the aberrant proliferation of GC cells through the regulation of p21, c-Myc, and Ki67. This may be the mechanism through which *HOXA5* exerts its anti-tumor effects on GC. Finally, nude mouse models also verified that *HOXA5* suppressed the proliferation of GC cells *in vivo*.

In conclusion, in this study, we demonstrated that *HOXA5* was a tumor suppressor gene and was decreased in GC. Its underexpression may be used as a direct prognostic indicator of a negative outcome. Our research may provide an opportunity for developing a novel therapeutic target as well as a prognostic marker in GC.

#### Acknowledgements

Not applicable.

#### Funding

This study was supported by the National Key Clinical Specialties Construction Program of China [grant no. (2012)649].

#### Availability of data and materials

All data generated or analyzed during this study are included in this published article and are freely available to any researchers.

#### Authors' contributions

ZW conceived and designed the experiments; XP, LZ and AC performed the experiments and analyzed the data; and XP wrote the manuscript. All authors have read and approved the final manuscript.

#### Ethics approval and consent to participate

The use of human tissue samples and experimental protocols were approved by the Medical Ethics Review Committee of the First Affiliated Hospital of Chongqing Medical University and written informed consent was obtained from all patients. The animal experimental protocols were approved by the Medical Ethics Review Committee of the First Affiliated Hospital of Chongqing Medical University.

#### Patient consent for publication

Not applicable.

#### Competing interests

The authors declare that they have no competing interests.

#### References

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A: Global cancer statistics, 2012. *CA Cancer J Clin* 65: 87-108, 2015.
2. Jiang H, Shah S and Hilt DC: Organization, sequence, and expression of the murine S100 beta gene. Transcriptional regulation by cell type-specific cis-acting regulatory elements. *J Biol Chem* 268: 20502-20511, 1993.
3. Boucherat O, Montaron S, Bérubé-Simard FA, Aubin J, Philippidou P, Wellik DM, Dasen JS and Jeannotte L: Partial functional redundancy between *Hoxa5* and *Hoxb5* paralog genes during lung morphogenesis. *Am J Physiol Lung Cell Mol Physiol* 304: L817-L830, 2013.
4. Ordóñez-Morán P, Dafflon C, Imajo M, Nishida E and Huelsenken J: *HOXA5* counteracts stem cell traits by inhibiting wnt signaling in colorectal cancer. *Cancer Cell* 28: 815-829, 2015.
5. Chen H, Zhang H, Lee J, Liang X, Wu X, Zhu T, Lo PK, Zhang X and Sukumar S: *HOXA5* acts directly downstream of retinoic acid receptor beta and contributes to retinoic acid-induced apoptosis and growth inhibition. *Cancer Res* 67: 8007-8013, 2007.
6. Wang CC, Su KY, Chen HY, Chang SY, Shen CF, Hsieh CH, Hong QS, Chiang CC, Chang GC, Yu SL and Chen JJ: *HOXA5* inhibits metastasis via regulating cytoskeletal remodelling and associates with prolonged survival in non-small-cell lung carcinoma. *PLoS One* 10: e124191, 2015.
7. Loh M, Liem N, Vaithilingam A, Lim PL, Sapari NS, Elahi E, Mok ZY, Cheng CL, Yan B, Pang B, *et al*: DNA methylation subgroups and the CpG island methylator phenotype in gastric cancer: A comprehensive profiling approach. *BMC Gastroenterol* 14: 55, 2014.
8. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2<sup>-</sup>(delta delta C(T)) method. *Methods* 25: 402-408, 2001.
9. Tang Z, Li C, Kang B, Gao G, Li C and Zhang Z: GEPIA: A web server for cancer and normal gene expression profiling and interactive analyses. *Nucleic Acids Res* 45: W98-W102, 2017.
10. Feng QY, Hu ZX, Song XL and Pan HW: Aberrant expression of genes and proteins in pterygium and their implications in the pathogenesis. *Int J Ophthalmol* 10: 973-981, 2017.
11. Marhaba R, Klingbeil P, Nuebel T, Nazarenko I, Buechler MW and Zoeller M: CD44 and EpCAM: Cancer-initiating cell markers. *Curr Mol Med* 8: 784-804, 2008.
12. Simon T, Gagliano T and Giamas G: Direct effects of anti-angiogenic therapies on tumor cells: VEGF signaling. *Trends Mol Med* 23: 282-292, 2017.
13. Chen Y, Pan K, Wang P, Cao Z, Wang W, Wang S, Hu N, Xue J, Li H, Jiang W, *et al*: HBPI-mediated regulation of p21 protein through the Mdm2/p53 and TCF4/EZH2 pathways and its impact on cell senescence and tumorigenesis. *J Biol Chem* 291: 12688-12705, 2016.
14. Gupta A, Kaur CD, Jangdey M and Saraf S: Matrix metalloproteinase enzymes and their naturally derived inhibitors: Novel targets in photocarcinoma therapy. *Ageing Res Rev* 13: 65-74, 2014.
15. Spitzwieser M, Pirker C, Koblmüller B, Pfeiler G, Hacker S, Berger W, Heffeter P and Cichna-Markl M: Promoter methylation patterns of ABCB1, ABCC1 and ABCG2 in human cancer cell lines, multidrug-resistant cell models and tumor, tumor-adjacent and tumor-distant tissues from breast cancer patients. *Oncotarget* 7: 73347-73369, 2016.
16. Zhang ML, Nie FQ, Sun M, Xia R, Xie M, Lu KH and Li W: *HOXA5* indicates poor prognosis and suppresses cell proliferation by regulating p21 expression in non small cell lung cancer. *Tumour Biol* 36: 3521-3531, 2015.
17. Ilson DH: Angiogenesis in gastric cancer: Hitting the target? *Lancet* 383: 4-6, 2014.

18. Mikhail S, Albanese C and Pishvaian MJ: Cyclin-dependent kinase inhibitors and the treatment of gastrointestinal cancers. *Am J Pathol* 185: 1185-1197, 2015.
19. Okimoto RA, Breitenbuecher F, Olivas VR, Wu W, Gini B, Hofree M, Asthana S, Hrustanovic G, Flanagan J, Tulpule A, *et al*: Inactivation of Capicua drives cancer metastasis. *Nat Genet* 49: 87-96, 2017.
20. Wang B, Chen Q, Cao Y, Ma X, Yin C, Jia Y, Zang A and Fan W: LGR5 is a gastric cancer stem cell marker associated with stemness and the EMT signature genes NANOG, NANOGP8, PRRX1, TWIST1, and BMI1. *PLoS One* 11: e168904, 2016.
21. Raman V, Martensen SA, Reisman D, Evron E, Odenwald WF, Jaffee E, Marks J and Sukumar S: Compromised HOXA5 function can limit p53 expression in human breast tumours. *Nature* 405: 974-978, 2000.
22. Nieto MA: Context-specific roles of EMT programmes in cancer cell dissemination. *Nat Cell Biol* 19: 416-418, 2017.
23. Aubin J, Déry U, Lemieux M, Chailier P and Jeannotte L: Stomach regional specification requires Hoxa5-driven mesenchymal-epithelial signaling. *Development* 129: 4075-4087, 2002.
24. Teo WW, Merino VF, Cho S, Korangath P, Liang X, Wu RC, Neumann NM, Ewald AJ and Sukumar S: HOXA5 determines cell fate transition and impedes tumor initiation and progression in breast cancer through regulation of E-cadherin and CD24. *Oncogene* 35: 5539-5551, 2016.
25. Wang Y, Xu L and Jiang L: MiR-1271 promotes non-small-cell lung cancer cell proliferation and invasion via targeting HOXA5. *Biochem Biophys Res Commun* 458: 714-719, 2015.
26. Yang HW, Chung M, Kudo T and Meyer T: Competing memories of mitogen and p53 signalling control cell-cycle entry. *Nature* 549: 404-408, 2017.
27. Ratti S, Ramazzotti G, Faenza I, Fiume R, Mongiorgi S, Billi AM, McCubrey JA, Suh PG, Manzoli L, Cocco L and Follo MY: Nuclear inositol signaling and cell cycle. *Adv Biol Regul* 67: 1-6, 2018.
28. Waldman T: The inaugural use of gene editing for the study of tumor suppressor pathways in human cells-p21WAF1/CIP1. *Cancer Res* 76: 4598-4601, 2016.
29. LaBaer J, Garrett MD, Stevenson LF, Slingerland JM, Sandhu C, Chou HS, Fattaey A and Harlow E: New functional activities for the p21 family of CDK inhibitors. *Genes Dev* 11: 847-862, 1997.
30. Feng F, Ren Q, Wu S, Saeed M and Sun C: Hoxa5 increases mitochondrial apoptosis by inhibiting Akt/mTORC1/S6K1 pathway in mice white adipocytes. *Oncotarget* 8: 95332-95345, 2017.



This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.